

Amendments to the Claims

This listing of claims will replace all prior version, and listings, of claims in the application:

Listing of Claims:

Claims 1-44. (cancelled)

45. (currently amended) A recombinant polynucleotide comprising a nucleotide sequence encoding a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal fused β -amyloid peptide ~~polypeptide~~, wherein the C-terminal β -amyloid peptide ~~polypeptide~~, when not fused to the rubredoxin constituent, is insoluble or forms inclusion bodies in a recombinant expression system.

46. (currently amended) The recombinant polynucleotide of claim 45 wherein the N-terminal rubredoxin constituent is cleavably linked to the C-terminal fused β -amyloid peptide ~~polypeptide~~.

47. (currently amended) The recombinant polynucleotide of claim 45 wherein the rubredoxin fusion protein further comprises an intervening spacer region positioned between the N-terminal rubredoxin constituent and the C-terminal fused β -amyloid peptide ~~polypeptide~~.

48. (previously presented) The recombinant polynucleotide of claim 47 wherein the intervening spacer region comprises at least one component selected from the group consisting of a proteolytic cleavage site and an affinity purification sequence.

49. (currently amended) A recombinant polynucleotide comprising a nucleotide sequence encoding a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal fused β -amyloid peptide ~~polypeptide~~, wherein the fusion protein binds a divalent cation and is chromogenic.

50. (currently amended) An expression vector comprising:
a nucleotide sequence encoding rubredoxin or a biologically active analogue, fragment, or modification thereof and a β -amyloid peptide; an intervening nucleotide sequence encoding a spacer region; and a multiple cloning region comprising at least one restriction endonuclease recognition site.

51. (previously presented) The expression vector of claim 50 wherein the intervening nucleotide sequence comprises all or a portion of the multiple cloning region.

52. (previously presented) The expression vector of claim 51 which is pRUBEX3, wherein pRUBEX3 comprises a nucleotide sequence encoding an affinity tag having at least one amino acid sequence selected from the group consisting of His-His-His-His-His-His (SEQ ID NO:4) and His-Gly-Leu-His (SEQ ID NO:7).

53. (previously presented) The expression vector of claim 50 wherein the intervening nucleotide sequence encodes at least one of a proteolytic cleavage site and an affinity purification sequence.

54. (currently amended) An expression vector comprising a promoter operably linked to a nucleotide sequence encoding a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal fused β -amyloid peptide ~~polypeptide~~, wherein the C-terminal β -amyloid peptide ~~polypeptide~~, when not fused to the rubredoxin constituent, is insoluble or forms inclusion bodies in a recombinant expression system.

55. (currently amended) An expression vector comprising a promoter operably linked to a nucleotide sequence encoding a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal fused β -amyloid peptide ~~polypeptide~~, wherein the fusion protein binds a divalent cation and is chromogenic.

56. (currently amended) A host cell transformed with an expression vector comprising a recombinant polynucleotide comprising a nucleotide sequence encoding a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal fused β -amyloid peptide ~~polypeptide~~, wherein the C-terminal β -amyloid peptide ~~polypeptide~~, when not fused to the rubredoxin constituent, is insoluble or forms inclusion bodies in a recombinant expression system.

57. (previously presented) The host cell of claim 56 which is a bacterial cell.

58. (currently amended) A host cell transformed with an expression vector comprising a recombinant polynucleotide comprising a nucleotide sequence encoding a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal

fused β -amyloid peptide ~~polypeptide~~, wherein the fusion protein binds a divalent cation and is chromogenic.

59. (previously presented) The host cell of claim 58 which is a bacterial cell.

60. (currently amended) A method for making a rubredoxin fusion protein comprising:

(a) introducing into a host cell a recombinant polynucleotide comprising a nucleotide sequence encoding a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal fused β -amyloid peptide ~~polypeptide~~; and

(b) expressing the fusion protein in the host cell, wherein the fusion protein binds a divalent cation and is chromogenic.

61. (previously presented) The method of claim 60 wherein the host cell contains or is supplied with at least one isotopically labeled amino acid or precursor compound, wherein the fusion protein expressed in the host cell in step (b) is isotopically labeled.

62. (previously presented) The method of claim 61 wherein the host cell is an amino acid auxotroph.

63. (previously presented) The method of claim 61 wherein the fused polypeptide is isotopically labeled with at least one of ^3H , ^{35}S , ^{13}C , or ^{15}N .

64. (cancelled)

65. (previously presented) The method of claim 60 further comprising (c) removing the fusion protein from the host cell.

66. (previously presented) The method of claim 65 further comprising (d) purifying the fusion protein.

67. (previously presented) The method of claim 66 wherein step (d) comprises visually tracking the location of the fusion protein.

68. (currently amended) The method of claim 66 wherein step (d) ~~comprising~~ comprises purifying the fusion protein using reverse phase chromatography at temperatures between about 45°C and about 65°C.

69. (currently amended) The method of claim 66 further comprising (e) cleaving the fusion protein to yield the rubredoxin constituent and the β -amyloid peptide ~~polypeptide~~.

70. (currently amended) The method of claim 69 further comprising (f) purifying the β -amyloid peptide ~~polypeptide~~ using reverse phase chromatography at temperatures between about 45°C and about 65°C.

71. (currently amended) A method for making a polypeptide ~~which, when not fused to a rubredoxin constituent, is insoluble or forms inclusion bodies in a recombinant expression system, the method~~ comprising:

(a) introducing into a host cell a recombinant polynucleotide comprising a nucleotide sequence encoding a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal fused β -amyloid peptide polypeptide, wherein the C-terminal β -amyloid peptide polypeptide, when not fused to the rubredoxin constituent, is insoluble or forms inclusion bodies in a recombinant expression system; and

(b) expressing the fusion protein in the host cell.

72. (previously presented) The method of claim 71 wherein the host cell contains or is supplied with at least one isotopically labeled amino acid or precursor compound, wherein the fused protein expressed in the host cell in step (b) is isotopically labeled.

73. (previously presented) The method of claim 72 wherein the host cell is an amino acid auxotroph.

74. (previously presented) The method of claim 72 wherein the fused protein is isotopically labeled with at least one of ^3H , ^{35}S , ^{13}C , or ^{15}N .

75. (cancelled)

76. (previously presented) The method of claim 71 further comprising (c) removing the fusion protein from the host cell.

77. (previously presented) The method of claim 76 further comprising (d) purifying the fusion protein.

78. (previously presented) The method of claim 77 wherein step (d) comprises visually tracking the location of the fusion protein.

79. (currently amended) The method of claim 77 wherein step (d) comprises purifying the fusion protein ~~is purified~~ using reverse phase chromatography at temperatures between about 45°C and about 65°C.

80. (currently amended) The method of claim 77 further comprising (e) cleaving the fusion protein to yield the rubredoxin constituent and the β -amyloid peptide ~~polypeptide~~.

81. (currently amended) The method of claim 80 further comprising (f) purifying the β -amyloid peptide ~~polypeptide~~ using reverse phase chromatography at temperatures about 45°C and about 65°C.

82. (currently amended) A method for making a rubredoxin- β -amyloid fusion protein comprising:

(a) introducing into a host cell a recombinant polynucleotide comprising a nucleotide sequenced encoding a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal fused β -amyloid peptide, wherein the host cell contains or is supplied with at least one isotopically labeled amino acid or precursor compound; and

(b) expressing a rubredoxin- β -amyloid fusion protein in the host cell wherein the fused β -amyloid peptide is uniformly isotopically labeled-; wherein said rubredoxin- β -amyloid fusion protein is soluble in a recombinant expression system.

83. (previously presented) The method of claim 82 wherein the rubredoxin β -amyloid fusion protein is uniformly labeled with at least one of ^3H , ^{13}C , ^{35}S and ^{15}N .

84. (previously presented) The method of claim 82 further comprising (c) removing the rubredoxin β -amyloid fusion protein from the host cell and (d) purifying the rubredoxin- β -amyloid fusion protein using reverse phase chromatography at temperatures between about 45°C and about 65°C .

85. (previously presented) The method of claim 82 further comprising cleaving the rubredoxin- β -amyloid fusion protein to yield the rubredoxin constituent and the β -amyloid peptide.

86. (previously presented) The method of claim 85 further comprising purifying the β -amyloid peptide using reverse phase chromatography at temperatures between about 45°C and about 65°C .

87. (currently amended) A method for making a rubredoxin- β -amyloid fusion protein comprising:

(a) introducing into a host cell a recombinant polynucleotide comprising a nucleotide sequence encoding a rubredoxin fusion protein comprising an N-terminal rubredoxin constituent and a C-terminal fused β -amyloid peptide, wherein the host cell contains or is supplied with at least one isotopically labeled amino acid or precursor compound selected from the group consisting of ^{35}S -methionine and an ^3H , ^{13}C , or ^{15}N -labeled precursor compound; and

(b) expressing a rubredoxin- β -amyloid fusion protein in the host cell wherein the fused β -amyloid peptide is uniformly labeled with at least one of ^3H , ^{13}C , ^{35}S and ^{15}N ; wherein said rubredoxin- β -amyloid fusion protein is soluble in a recombinant expression system.

Claims 88 - 111 (canceled)

112. (currently amended) A vaccine comprising: ~~at least one component selected from the group consisting of:~~ a polynucleotide comprising a nucleotide sequence encoding a rubredoxin fusion protein, wherein the rubredoxin fusion protein comprises an N-terminal rubredoxin constituent and a C-terminal fused β -amyloid peptide ~~polypeptide~~; and a pharmaceutically acceptable carrier.

113. (currently amended) The vaccine of claim 112 wherein the N-terminal rubredoxin constituent is directly linked to the C-terminal fused β -amyloid peptide ~~polypeptide~~.

114. (previously presented) The vaccine of claim 112 further comprising an adjuvant.